flanked by AAV inverted terminal repeats, rep and cap genes, or helper virus genes. In one embodiment, the host cell comprises a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, rep and cap genes, and helper virus genes. In one embodiment, the host cell comprises one or more AAV genes stably integrated into the host cell's genome.

[0009] It is contemplated that the helper virus may be any virus capable of allowing AAV contained in a host cell to enter the infections phase, for example adenovirus, herpes simplex virus, papilloma virus, or baculovirus. In one embodiment, the helper virus is adenovirus subtype 5 (Ad5).

[0010] In certain embodiments, the host cell will be capable of producing both rAAV and helper virus. A host cell is capable of producing rAAV or AV if, in the absence of intervention and given appropriate culture conditions, the cell will produce viral particles, whether or not the particles are released into the cell culture media. A host cell may be capable of producing a virus because it was infected by a live virus, or because it was transfected with viral genes that may exist in the cell transiently, for example, on a plasmid or other extrachromosomal body, or be permanently integrated into the host cell genome. It is contemplated host cells transiently transfected with one or more plasmids containing AAV inverted terminal repeats, rep and cap genes, and infected by live helper virus may be considered capable of producing both rAAV and helper virus. It is further contemplated that host cells containing AAV inverted terminal repeats, rep and cap genes integrated in the host cell's chromosomes and infected with a live helper virus will be considered capable of producing both rAAV and helper virus.

[0011] In other embodiments, the host cell is inoculated with non-replicating helper virus. In this situation, the host cell is capable of producing rAAV but not capable of producing helper virus, and thus the beneficial effects of increased osmolality on helper virus production will not occur, however the beneficial effect of increased rAAV production will still be realized.

[0012] In other aspects, the invention provides a rAAV produced by any of the contemplated methods, a composition comprising a rAAV produced by any of the contemplated methods, or a cell culture system comprising a host cell capable of producing both rAAV and helper virus and cell culture media with an osmolality of 360 mOsm/kg or higher.

[0013] These and other aspects and features of the invention are described in the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The foregoing and other objects, features and advantages of the invention will become apparent from the following description of preferred embodiments, as illustrated in the accompanying drawings. Like referenced elements identify common features in the corresponding drawings.

[0015] FIG. **1**A depicts the effect of the addition of the ionic tonicifying agent NaCl on the production of extracellular rAAV and Ad5 in a HeLa producer cell line at 4 days post inoculation and infection. rAAV and Ad5 production was quantified by qPCR. Each value represents the mean of two independent experiments.

[0016] FIG. **1**B depicts the effect of the addition of the ionic tonicifying agent NaCl on the production of total rAAV and Ad5 in a HeLa producer cell line at 4 days post inoculation and infection. rAAV and Ad5 production was quantified by qPCR. Each value represents the mean of two independent experiments.

[0017] FIG. **2**A depicts the effect of the addition of the non-ionic tonicifying agent sucrose on the production of extracellular rAAV and Ad5 in a HeLa producer cell line at 4 days post inoculation and infection. rAAV and Ad5 production was quantified by qPCR. Each value represents the mean of two independent experiments.

[0018] FIG. **2**B depicts the effect of the addition of the non-ionic tonicifying agent sucrose on the production of total rAAV and Ad5 in a HeLa producer cell line at 4 days post inoculation and infection. rAAV and Ad5 production was quantified by qPCR. Each value represents the mean of two independent experiments.

DETAILED DESCRIPTION

[0019] The invention is based, in part, upon the discovery that the production of rAAV and helper virus in a host cell can be optimized by increasing the osmolality of the culture media through the use of a tonicifying agent, such as NaCl or sucrose. In one aspect, the invention provides a method for producing rAAV using a helper virus, the method comprising incubating a host cell capable of producing both rAAV and helper virus in a cell culture medium containing one or more tonicifying agents and having an osmolality of 360 mOsm/kg or higher at the start of the incubation period. In another aspect, the invention provides a method for decreasing the amount of helper virus produced during the production of rAAV by incubating a host cell in a cell culture medium containing one or more tonicifying agents and having an osmolality of 360 mOsm/kg or higher at the start of the incubation period. In yet another aspect, the invention provides a method for increasing the amount of rAAV produced by a host cell while simultaneously decreasing the amount of helper virus produced by a host cell, by incubating a host cell in a cell culture medium containing one or more tonicifying agents and having an osmolality of 360 mOsm/kg or higher at the start of the incubation period.

1. Adeno-Associated Virus

[0020] Adeno-associated virus (AAV) is a small, nonenveloped icosahedral virus of the genus Dependoparvovirus and family Parvovirus. AAV has a single-stranded linear DNA genome of approximately 4.7 kb. AAV includes numerous serologically distinguishable types including serotypes AAV-1 to AAV-12, as well as more than 100 serotypes from nonhuman primates (See, e.g., Srivastava, J. Cell Biochem., 105(1): 17-24 (2008), and Gao et al., J. Virol., 78(12), 6381-6388 (2004)). Any AAV type may be used in the methods of the present invention. AAV is capable of infecting both dividing and quiescent cells of several tissue types, with different AAV serotypes exhibiting different tissue tropism. AAV is non-autonomously replicating, and has a life cycle with a latent phase and an infectious phase. In the latent phase, after a cell is infected with an AAV, the AAV site-specifically integrates into the host's genome as a provirus. The infectious phase does not occur unless the cell is also infected with a helper virus (for